

# Soil Bacteria Confer Plant Salt Tolerance by Tissue-Specific Regulation of the Sodium Transporter *HKT1*

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Submitted 4 November 2007. Accepted 6 February 2008.

**Elevated sodium (Na<sup>+</sup>) decreases plant growth and, thereby, agricultural productivity. The ion transporter high-affinity K<sup>+</sup> transporter (HKT)1 controls Na<sup>+</sup> import in roots, yet dysfunction or overexpression of *HKT1* fails to increase salt tolerance, raising questions as to *HKT1*'s role in regulating Na<sup>+</sup> homeostasis. Here, we report that tissue-specific regulation of *HKT1* by the soil bacterium *Bacillus subtilis* GB03 confers salt tolerance in *Arabidopsis thaliana*. Under salt stress (100 mM NaCl), GB03 concurrently down- and upregulates *HKT1* expression in roots and shoots, respectively, resulting in lower Na<sup>+</sup> accumulation throughout the plant compared with controls. Consistent with *HKT1* participation in GB03-induced salt tolerance, GB03 fails to rescue salt-stressed *athkt1* mutants from stunted foliar growth and elevated total Na<sup>+</sup> whereas salt-stressed Na<sup>+</sup> export mutants *sos3* show GB03-induced salt tolerance with enhanced shoot and root growth as well as reduced total Na<sup>+</sup>. These results demonstrate that tissue-specific regulation of *HKT1* is critical for managing Na<sup>+</sup> homeostasis in salt-stressed plants, as well as underscore the breadth and sophistication of plant–microbe interactions.**

Soil salinity is a major constraint to modern agriculture, with approximately 20% of once-irrigated land worldwide presently salt contaminated (Frommer et al. 1999). For most trees and crop plants that are salt sensitive, elevated Na<sup>+</sup> imposes toxic effects by perturbing potassium (K<sup>+</sup>)-dependent processes, inducing deleterious protein conformations, and causing osmotic stress that causes growth inhibition and, ultimately, cell death (Chinnusamy et al. 2006; Greenway and Munns 1980; Wyn Jones 1981). To avoid accumulating toxic Na<sup>+</sup> levels in the shoots, plants must take up no more than 3% of the Na<sup>+</sup> present in the rhizosphere (Munns et al. 1999). For salt-stressed plants, restricting Na<sup>+</sup> uptake and shoot Na<sup>+</sup> accumulation is critical for minimizing salt phytotoxicity.

High-affinity K<sup>+</sup> transporters (HKT) mediate Na<sup>+</sup> transport in higher plants, including *Arabidopsis*, wheat, and rice (Platten et al. 2006). *HKT1* originally was sequenced from wheat roots and identified as HKT (Schachtman and Schroeder 1994). Yeast expression of wheat *HKT1* revealed that, at physiologically detrimental Na<sup>+</sup> concentrations, *HKT1* switches from an HKT to a low-affinity Na<sup>+</sup> transporter (Rubio et al. 1995). Studies in heterologous expression systems and in planta have

established *HKT1* as a Na<sup>+</sup> transporter that controls Na<sup>+</sup> uptake (Haro et al. 2005; Mäser et al. 2002a; Rubio et al. 1995; Rus et al. 2001, 2004; Schachtman and Schroeder 1994; Sunarpi et al. 2005; Uozumi et al. 2000). In *Arabidopsis*, *HKT1* functions as a negative regulator of K<sup>+</sup> nutrition rather than as a K<sup>+</sup> uptake system (Rus et al. 2004), whereas *AtHKT1* expressed in *Xenopus* oocytes exhibits selective transport of Na<sup>+</sup> but not K<sup>+</sup> (Uozumi et al. 2000). In addition to regulation of Na<sup>+</sup> influx in roots (Haro et al. 2005; Laurie et al. 2002; Rus et al. 2001), *HKT1* and other HKT proteins are proposed to function in shoots to retrieve Na<sup>+</sup> from the xylem, thereby facilitating shoot-to-root Na<sup>+</sup> recirculation, as demonstrated in *Arabidopsis* (Berthomieu et al. 2003; Davenport et al. 2007; Mäser et al. 2002a; Sunarpi et al. 2005), rice (Garcia-deblas et al. 2003; Ren et al. 2005), and wheat (Byrt et al. 2007; Huang et al. 2006; James et al. 2006). Being a driver for Na<sup>+</sup> influx and recirculation, *HKT1* has unexpectedly been an elusive target for engineering Na<sup>+</sup> regulation in plants, because both loss-of-function and overexpression of *HKT1* disturb ion status and increase salt sensitivity (Mäser et al. 2002a; Rus et al. 2004).

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil microorganisms that colonize roots and stimulate plant growth. Such bacteria have been applied to a wide range of agricultural crops for the purpose of growth enhancement, including increased seed germination, plant weight, harvest yields, and disease resistance (Kloepper et al. 1980, 1991, 1999). Under non-salt-stress conditions, PGPR colonization is proposed to trigger growth by bacterial synthesis of the plant hormones indole-3-acetic acid, cytokinin, and gibberellins, as well as by increased mineral and nitrogen availability in the soil (Glick 1999; Lin et al. 1983; Loper and Schroth 1986; MacDonald et al. 1986; Timmusk et al. 1999). In the absence of physical contact with plant roots, blends of volatile chemicals emitted from specific strains of PGPR trigger growth promotion in *Arabidopsis* (Paré et al. 2005; Ryu et al. 2003, 2004). *Bacillus subtilis* GB03, a commercially available soil symbiont, is one such strain that emits a complex blend of volatile components that activates plant growth promotion. A bouquet of over 25 bacterial volatile odors has been identified that trigger differential expression of approximately 600 *Arabidopsis* transcripts related to cell wall modifications, primary and secondary metabolism, stress responses, hormone regulation, and other expressed proteins (Frag et al. 2006; Ryu et al. 2003; Zhang et al. 2007). In addition, whole-plant *HKT1* transcript levels are reduced in plants grown under low Na<sup>+</sup> conditions, presenting a possible regulatory role of GB03 in increasing salt tolerance (Zhang et al. 2007). In this study, the potential of GB03 to increase plant re-

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sistance to salt stress by regulating *HKT1* gene expression is investigated. Here, we monitor plant salt tolerance and tissue-specific *HKT1* gene expression with *Arabidopsis* exposure to GB03 volatiles. Mutant studies allow for assigning an *HKT1* role in GB03-triggered plant salt tolerance.

## RESULTS

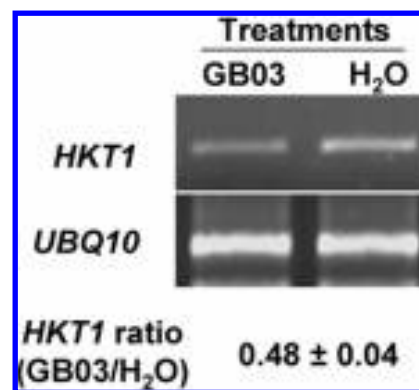
### GB03 induces *HKT1*-dependent plant salt tolerance.

With elevated NaCl (100 mM) in the growth medium, *Arabidopsis* (*Col-0*) plants exposed to GB03 volatiles show robust foliar growth (Fig. 1A) compared with water controls. Fourteen days after treatments, GB03-exposed plants clearly exhibit enhanced shoot growth (Fig. 1B) as well as root growth (Fig. 1C) on medium with 100 mM NaCl, demonstrating the capacity of GB03 to confer plant salt tolerance. The abundance of *HKT1* transcripts in whole plants grown with 100 mM NaCl decreases to 48% 4 days postexposure to GB03 (Fig. 2). Interestingly, ubiquitous dysfunction of *Arabidopsis HKT1* has been shown to cause higher Na<sup>+</sup> accumulation in shoots and, thereby, foliar Na<sup>+</sup> hypersensitivity with 100 mM NaCl (Horie et al. 2006; Mäser et al. 2002a). Although GB03 concomitantly reduces *HKT1* expression and enhances shoot growth in wild-type (WT) *Arabidopsis*, *hkt1* mutants exhibited stunted shoot growth under salt stress with or without GB03 present (Fig. 3A and B), indicating that *HKT1* is critical for GB03 to trigger tolerance in shoots of salt-stressed plants. Meanwhile, *hkt1* mutation results in greater root growth in salt-stressed plants, with the average root mass being 2.1 mg in *hkt1* (Fig. 3C) compared with 1.1 mg for WT plants (Fig. 1C), which is consistent with the observation that GB03-triggered reduction of *HKT1* correlates with greater root growth in salt-stressed

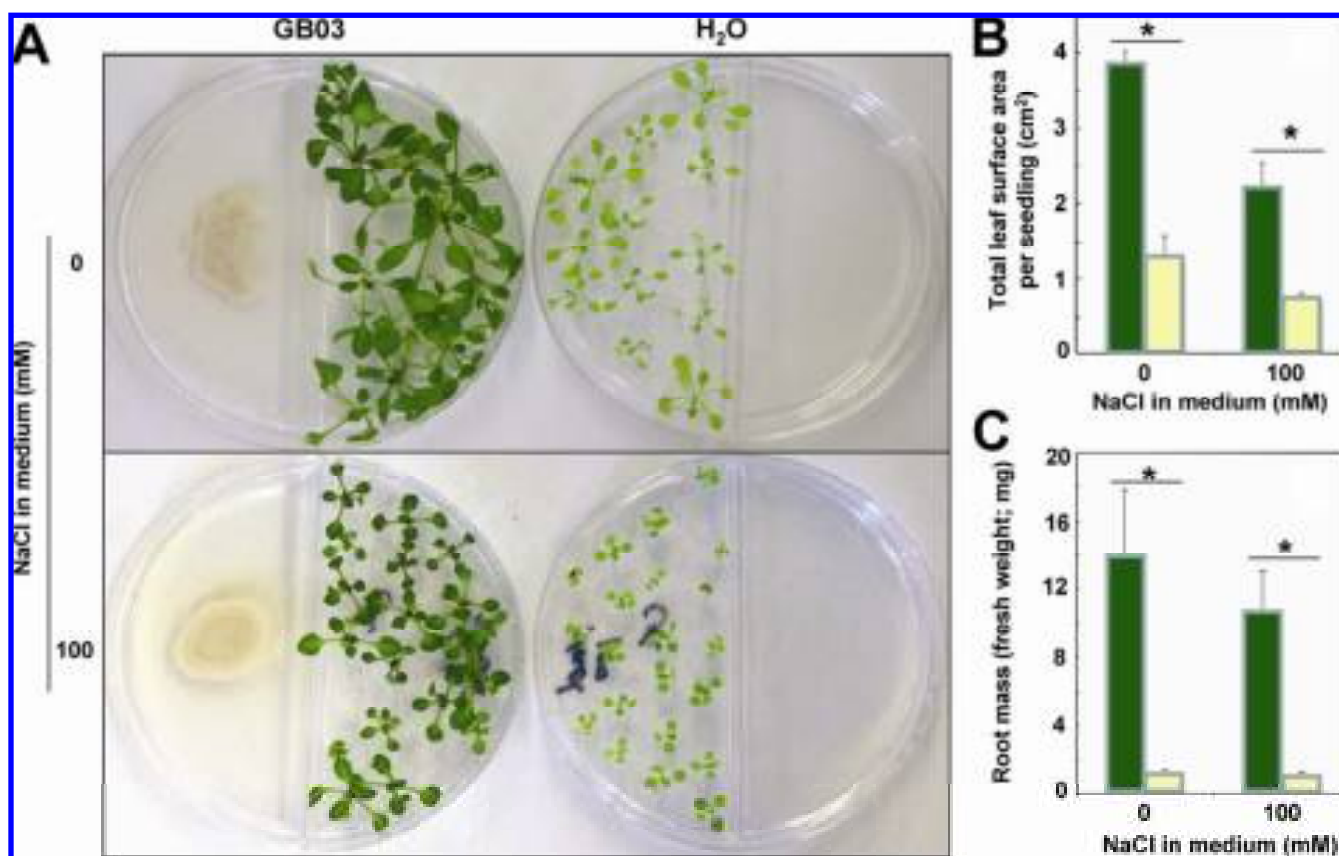
plants (Fig. 1C). Combined, these observations suggest that bacterial regulation of *HKT1* may not be uniform throughout the plant as in *hkt1*.

### Tissue-specific *Arabidopsis HKT1* regulation by GB03.

To characterize GB03 regulation, *HKT1* transcripts were monitored tissue specifically. GB03 exposure up- and down-regulates *HKT1* gene expression in shoots and roots, respectively, within 4 days (Fig. 4A and B). Twenty-four hours of bacteria exposure is sufficient to reduce root *HKT1* expression by approximately 50% compared with control plants, although



**Fig. 2.** *HKT1* transcript level in salt-stressed plants is decreased by GB03. Plants (*Col-0*) were grown in medium with 100 mM NaCl and harvested 4 days after exposure to GB03 or water control. Quantification of *HKT1* expression ratio (GB03/H<sub>2</sub>O) from reverse-transcriptase polymerase chain reaction results is shown; *UBQ10* is employed as an equal loading control ( $n = 3$ , mean  $\pm$  standard deviation).



**Fig. 1.** GB03 confers salt tolerance in *Arabidopsis* (*Col-0*). **A**, Representative images are depicted of plants with exposure to GB03 or water as control for 14 days. GB03 triggers enhancement of **B**, shoot growth as well as **C**, root growth. Green and yellow bars represent GB03 and water control treatments, respectively; an asterisk (\*) indicates a  $P \leq 0.05$  for treated versus controls ( $t$  test;  $n \geq 30$ , mean  $\pm$  standard deviation).

shoot *HKT1* transcript levels were not significantly different in GB03-exposed and control plants at that time. GB03-triggered induction of shoot *HKT1* expression shows a bell-shaped trend, with the maximum induction being more than fourfold after 3 days of treatments (Fig. 4A). Meanwhile, up to 70% reduction in root *HKT1* expression of GB03-exposed plants was observed compared with control plants (Fig. 4B). In light of the fact that *HKT1* controls  $\text{Na}^+$  uptake in roots as well as shoot-to-root  $\text{Na}^+$  recirculation (Berthomieu et al. 2003; Davenport et al. 2007; Laurie et al. 2002; Mäser et al. 2002a; Rus et al. 2001; Sunarpi et al. 2005), tissue-specific regulation of *HKT1* expression by GB03 suggests reduced  $\text{Na}^+$  uptake and enhanced shoot-to-root  $\text{Na}^+$  recirculation (Fig. 4C).

### **HKT1 mediates GB03-regulated $\text{Na}^+$ and $\text{K}^+$ homeostasis with salt stress.**

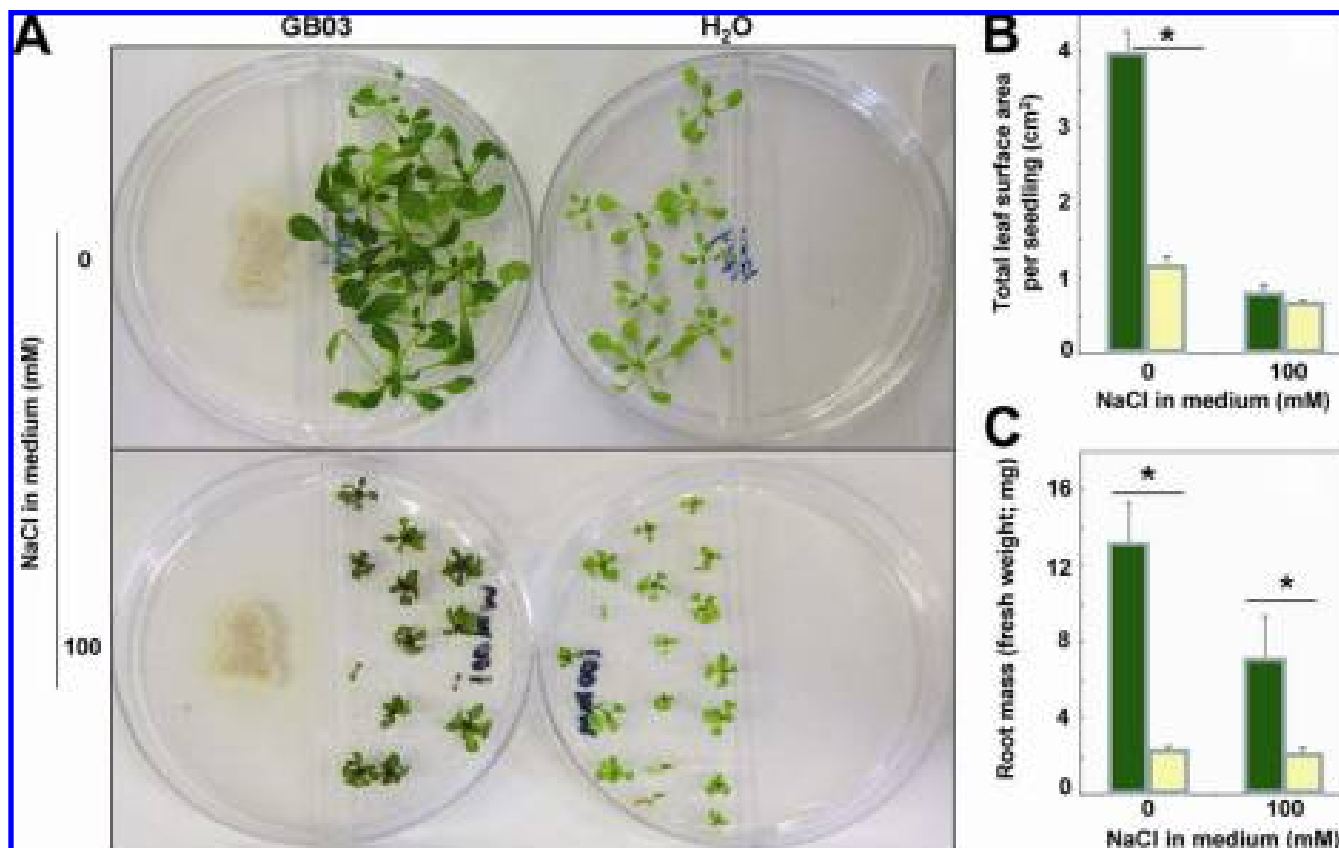
To test the hypothesis that tissue-specific regulation of *HKT1* is necessary and sufficient for GB03-induced plant salt tolerance,  $\text{Na}^+$  accumulation was examined in plants grown with 100 mM NaCl. Fourteen days postexposure, total  $\text{Na}^+$  accumulation in GB03-exposed plants was 54% of that in nonexposed plants (Fig. 5A), indicating reduced  $\text{Na}^+$  import, enhanced  $\text{Na}^+$  export, or both. In GB03-exposed roots,  $\text{Na}^+$  reduction followed lower *HKT1* expression coding for  $\text{Na}^+$  import (Fig. 5A and 4B); whereas, for GB03-exposed shoots,  $\text{Na}^+$  reduction was consistent with enhanced *HKT1* expression coding for  $\text{Na}^+$  recirculation. In addition, the *hkt1* mutation abrogated reduction of  $\text{Na}^+$  accumulation triggered by GB03 exposure in the whole plant as well as tissue specifically (Fig. 5B). Without shoot *HKT1* operative (*hkt1*), GB03 failed to reduce shoot  $\text{Na}^+$  levels. Assuming that shoot *HKT1* regulates shoot-to-root  $\text{Na}^+$  recirculation,

(Berthomieu et al. 2003; Davenport et al. 2007; Mäser et al. 2002a), the difference in shoot  $\text{Na}^+$  for mutant and WT lines (Fig. 5A and B) provides an approximation of  $\text{Na}^+$  recirculation. However, because significant differences in total  $\text{Na}^+$  between *hkt1* and WT lines with GB03 exposure do exist (80 versus 140 mg per gram of dry weight, respectively), unidentified factors such as reduced uploading of  $\text{Na}^+$  from roots to shoots may overestimate GB03-induced  $\text{Na}^+$  recirculation values.

In addition to reducing  $\text{Na}^+$  levels, GB03 exposure increases total  $\text{K}^+$  content in WT plants (Fig. 5C). Specifically,  $\text{K}^+$  accumulation in roots was increased more than twofold, while  $\text{K}^+$  level in shoots decreased by approximately 50% (Fig. 5C). In contrast, no difference of  $\text{K}^+$  level was observed in *hkt1* plants with or without GB03 exposure (Fig. 5D). Water-treated *hkt1* mutants exhibited higher  $\text{K}^+$  levels ( $74.0 \pm 1.8$  mg/g dry weight) than WT plants ( $24.2 \pm 0.5$  mg/g dry weight) (Fig. 5C and D), confirming that *HKT1* negatively regulates  $\text{K}^+$  accumulation. The negative role of *HKT1* in  $\text{K}^+$  accumulation is supported by the observations that higher *HKT1* expression is detected in shoots of GB03-exposed WT plants (Fig. 4A) and accumulate less  $\text{K}^+$  (Fig. 5C), whereas roots have lower *HKT1* expression (Fig. 4B) and accumulate more  $\text{K}^+$  (Fig. 5C). GB03 modulation of  $\text{Na}^+$  and  $\text{K}^+$  accumulation in salt-stressed WT plants resulted in an increased  $\text{K}^+/\text{Na}^+$  ratio from a whole-plant perspective and, specifically, in roots (Fig. 3E). No  $\text{K}^+/\text{Na}^+$  ratio difference was observed for *hkt1* plants with or without GB03 exposure (Fig. 5F).

### **GB03 induces salt tolerance in the $\text{Na}^+$ export mutant *sos3*.**

To determine whether  $\text{Na}^+$  export participates in GB03-triggered reduction of plant  $\text{Na}^+$  levels, GB03 effects were moni-



**Fig. 3.** The *hkt1* mutation disrupts GB03-triggered plant salt tolerance. **A**, Representative images are shown of plants with exposure to GB03 or water controls for 14 days. **B**, *HKT1* is not necessary for GB03-triggered growth promotion in plants without salt stress, whereas *hkt1* mutation abolishes plant salt tolerance induced by GB03. **C**, GB03 enhances root growth in *hkt1* mutants. Green and yellow bars represent GB03 and water control treatments, respectively; an asterisk (\*) indicates a  $P \leq 0.05$  for treated versus controls ( $t$  test;  $n \geq 30$ , mean  $\pm$  standard deviation).

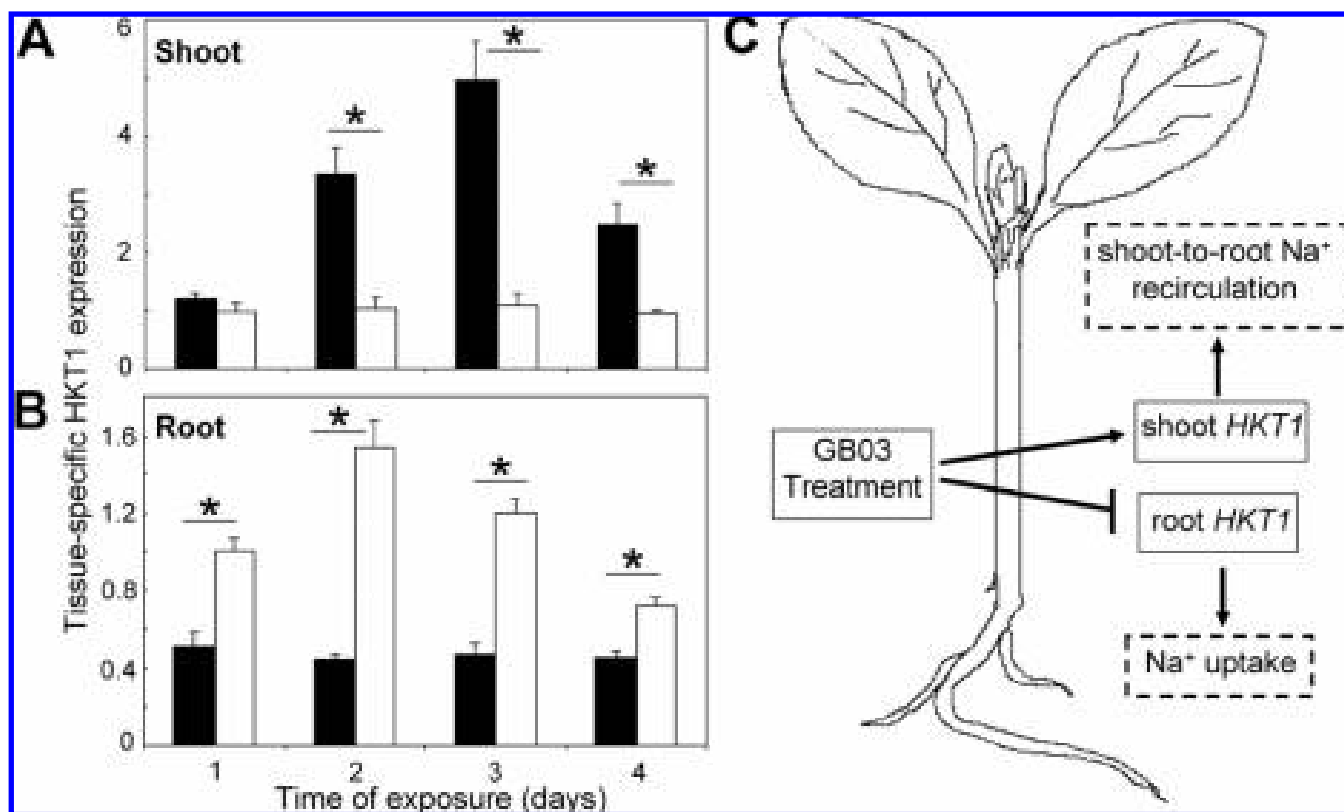
tored using an *Arabidopsis sos3* mutant line that is hypersensitive to 30 mM NaCl due to disruption in the SOS signaling pathway that regulates Na<sup>+</sup> efflux (Rus et al. 2001; Zhu 2002). Salt-stressed *sos3* plants concomitantly show less root- and shoot-growth inhibition with GB03 exposure (Fig. 6A through C). Moreover, 14 days postexposure, GB03 decreased total Na<sup>+</sup> level by 18% in *sos3* plants grown with 30 mM NaCl (Fig. 6D), suggesting that Na<sup>+</sup> accumulation can be reduced by GB03 independent of Na<sup>+</sup> export activation. Therefore, GB03-reduced total Na<sup>+</sup> accumulation (Fig. 3A) can be attributed, at least in part, to reduced Na<sup>+</sup> uptake. GB03-triggered decrease of Na<sup>+</sup> level was not observed in *sos3* roots (Fig. 6D). This may be due to defected Na<sup>+</sup> export by *sos3* mutation combined with enhanced shoot-to-root Na<sup>+</sup> recirculation triggered by GB03. Na<sup>+</sup> hypersensitivity of *sos3* plants correlates with reduced K<sup>+</sup> accumulation, resulting in root growth inhibition (Rus et al. 2001; Zhu et al. 1998). With GB03 exposure, K<sup>+</sup> content in *sos3* roots was increased by more than twofold (Fig. 6E). Thus, increased K<sup>+</sup> content in *sos3* roots is consistent with the suppression of salt-inhibited root growth.

## DISCUSSION

HKT1 is a focal determinant of Na<sup>+</sup> homeostasis. Although whole-plant genetic manipulation of *HKT1* has been unsuccessful to confer salt tolerance, herein we show that tissue-specific *HKT1* gene regulation by the soil bacterium GB03 effectively controls Na<sup>+</sup> homeostasis in salt-stressed plants. Mutant studies further confirmed the key role of *HKT1* tissue-specific regulation in GB03-triggered plant salt tolerance. In-

creasing evidence supports the dual role of HKT1 in regulating Na<sup>+</sup> homeostasis via Na<sup>+</sup> uptake and recirculation; therefore, observations in this study underscore the importance of tissue-specific investigation in understanding HKT1 activity and function. Such tissue-specific regulation of HKT1 by beneficial bacteria provides an alternative strategy for crop protection against high soil salinity.

Plants in saline environment can protect themselves from Na<sup>+</sup> toxicity through restricting Na<sup>+</sup> entry; excluding Na<sup>+</sup> from root cells; sequestering Na<sup>+</sup> into vacuoles; or retrieving Na<sup>+</sup> from the transpirational xylem stream for recirculation to roots (Chinnusamy et al. 2006). Na<sup>+</sup>/H<sup>+</sup> antiporters in the plasma membrane (*SOS1*) and in the tonoplast (*NHX1*) control Na<sup>+</sup> export and vacuolar Na<sup>+</sup> sequestration, respectively. Genetic engineering for plant salt tolerance through overexpression of these two genes has been straightforward and successful (Apse et al. 1999; Shi et al. 2003). In contrast, although HKT1 regulates Na<sup>+</sup> uptake as well as shoot-to-root Na<sup>+</sup> recirculation, ubiquitous alteration of *HKT1* gene expression has been unfavorable for conferring plant salt tolerance. Disruption of *HKT1* in *Arabidopsis* leads to shoot Na<sup>+</sup> hypersensitivity due to higher Na<sup>+</sup> accumulation in shoots (Mäser et al. 2002a; Rus et al. 2004); whereas overexpression of *HKT1* causes Na<sup>+</sup>-specific salt hypersensitivity and decreases K<sup>+</sup>/Na<sup>+</sup> ratios in roots due to reduced K<sup>+</sup> accumulation (Rus et al. 2004). In addition, dysfunction of *HKT1* has been reported to partially rescue Na<sup>+</sup> inhibition of root growth in *sos3* background (Rus et al. 2001); however, *hkt1 sos3* double mutants are still shoot salt sensitive, similar to the *hkt1* mutant, as a result of increased shoot Na<sup>+</sup> level (Horie et al. 2006). Because *sos3* and *hkt1* exhibit salt



**Fig. 4.** *HKT1* tissue-specific expression in salt-stressed *Col-0* is differentially regulated with GB03 exposure. Two-day-old wild-type seedlings are GB03 exposed and **A**, shoot as well as **B**, root *HKT1* is monitored over a 4-day period. The values detected by quantitative real-time polymerase chain reaction are relative to water-treated values observed on day 1; black and white bars represent GB03 and controls, respectively, with an asterisk (\*) indicating a  $P \leq 0.05$  for treated versus controls ( $t$  test;  $n = 3$ , mean  $\pm$  standard deviation). **C**, Model of *HKT1*-dependent Na<sup>+</sup> regulation by GB03. *HKT1* controls Na<sup>+</sup> import in roots (Laurie et al. 2002; Rus et al. 2001) as well as Na<sup>+</sup> transport from xylem vessels to phloem for shoot-to-root Na<sup>+</sup> recirculation (Berthomieu et al. 2003; Davenport et al. 2007; Mäser et al. 2002a; Sunarpi et al. 2005). Therefore, GB03-triggered reduction of root *HKT1* limits Na<sup>+</sup> entry into plants, whereas GB03-induction of shoot *HKT1* facilitates Na<sup>+</sup> retrieval from shoot to root.

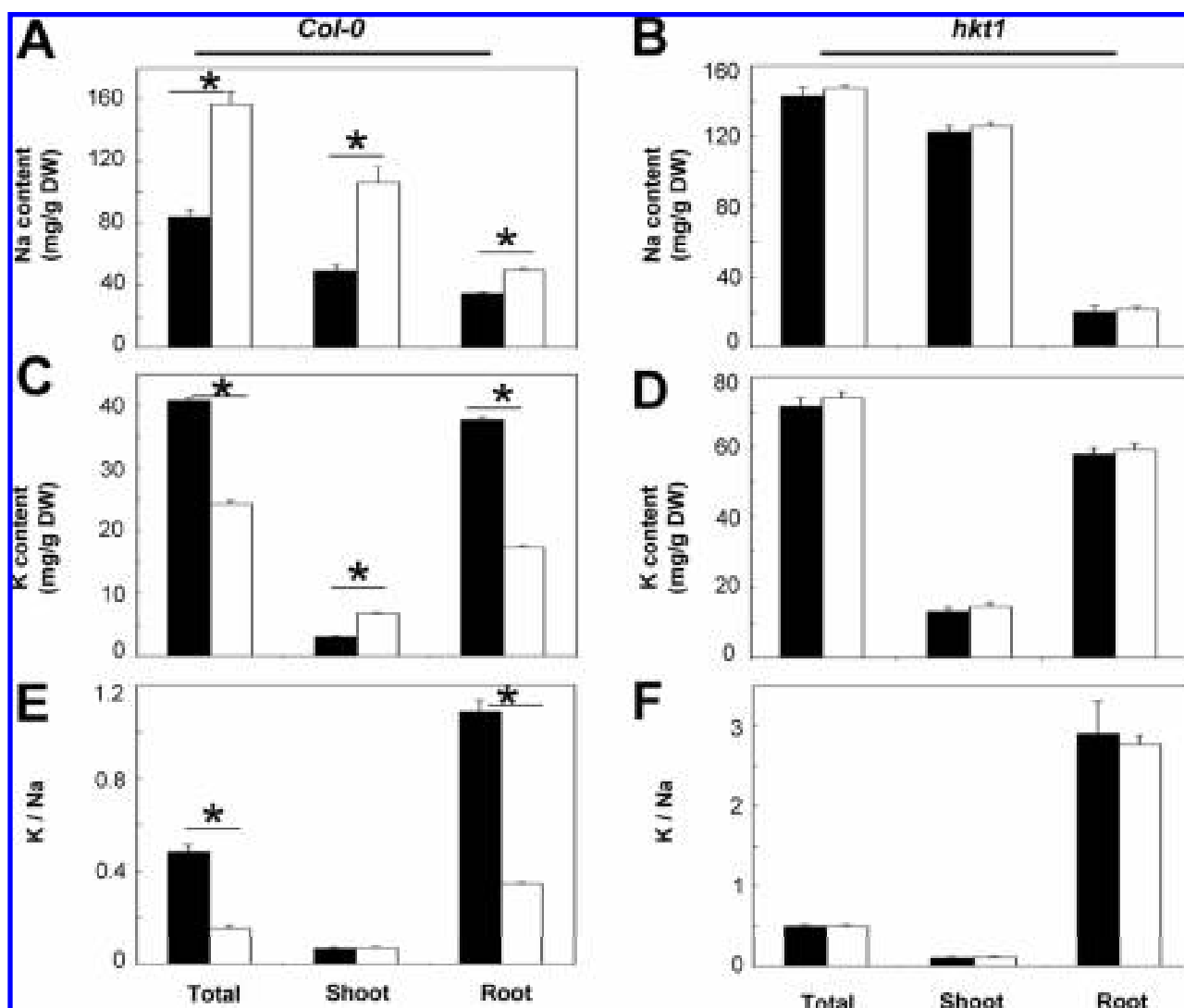
stress at different salt doses (30 and 100 mM NaCl, respectively) (Horie et al. 2006; Mäser et al. 2002a; Rus et al. 2001), individual salt-stress conditions were necessary for each mutant line to assay for GB03-induced salt tolerance and avoid salt toxicity.

Given the dual function of HKT1 in regulating Na<sup>+</sup> homeostasis, with Na<sup>+</sup> import into roots (Laurie et al. 2002; Rus et al. 2001) and Na<sup>+</sup> recirculation from the shoots (Berthomieu et al. 2003; Davenport et al. 2007; Mäser et al. 2002a; Sunarpi et al. 2005), tissue-specific regulation of *HKT1* expression appears to be critical for GB03-conferred salt tolerance in plants (Fig. 4C). Reduction of root *HKT1* limits Na<sup>+</sup> entry into plants, while induction of shoot *HKT1* facilitates Na<sup>+</sup> retrieval from shoot to root, thereby managing Na<sup>+</sup> equilibrium and conferring salt tolerance. Consistent with the upregulation of shoot *HKT1* expression that indicates enhanced shoot-to-root Na<sup>+</sup> recirculation, GB03-exposed plants accumulate less Na<sup>+</sup> in shoots compared with control plants. Meanwhile, the down-regulation of root *HKT1* correlates with reduced root as well as whole-plant Na<sup>+</sup> levels. Therefore, tissue-specific regulation is

here shown to be critical for plant salt tolerance conferred through manipulation of *HKT1* gene expression.

Bacterial regulation reduces whole-plant Na<sup>+</sup> accumulation as evidenced by lower Na<sup>+</sup> levels in both shoots and roots. Such reduction in Na<sup>+</sup> accumulation is unlikely to be dependent on activation of Na<sup>+</sup> efflux, because disruption of the SOS signaling pathway that regulate Na<sup>+</sup> efflux does not abolish the GB03-increased salt tolerance (Fig. 6). The key role of HKT1 in GB03-induced resistance to salt stress is further confirmed by *hkt1* mutant plants, which show higher shoot Na<sup>+</sup> level as well as Na<sup>+</sup> hypersensitivity in shoots than WT plants. GB03 is unable to reduce shoot Na<sup>+</sup> accumulation or to release the salt-stressed phenotype in shoots of *hkt1* plants, indicating that GB03-induced salt tolerance requires functional *HKT1*.

Toxic levels of Na<sup>+</sup> perturb K<sup>+</sup> homeostasis that is essential for plant metabolism (Elumalai et al. 2002; Maathuis and Sanders 1996; Rigas et al. 2001). Functional expression in heterologous systems revealed that wheat HKT1 mediates Na<sup>+</sup>-coupled K<sup>+</sup> transport, whereas *Arabidopsis* HKT1 transports only Na<sup>+</sup> (Rubio et al. 1995; Uozumi et al. 2000). The different



**Fig. 5.** GB03 decreases Na<sup>+</sup> accumulation and increases K<sup>+</sup> nutrient status under salt stress and functioning HKT1. **A**, Na<sup>+</sup> level is lower in 16-day-old wild-type shoots and roots with GB03 exposure for 14 days whereas **B**, *HKT1* dysfunction abolishes the GB03-triggered reduction of foliar Na<sup>+</sup> accumulation and reduces root Na<sup>+</sup> level compared with the wild type. Correlated with GB03-induced up- and downregulation of *HKT1* in shoots and roots, respectively, **C**, GB03-exposed plants accumulate less and more K<sup>+</sup> in shoots and roots, respectively, indicating a negative role of *HKT1* in K<sup>+</sup> uptake in the wild type. **D**, GB03 does not affect K<sup>+</sup> accumulation in *hkt1* mutants. **E**, As a result of regulating Na<sup>+</sup> and K<sup>+</sup> accumulation tissue specifically, GB03 increases K<sup>+</sup>/Na<sup>+</sup> ratios in roots but not in shoots of *Col-0*. **F**, K<sup>+</sup>/Na<sup>+</sup> ratios in *hkt1* plants are not affected by GB03 exposure. Black and white bars represent GB03 and water controls, respectively, with an asterisk (\*) indicating  $P \leq 0.05$  for treated versus controls ( $t$  test;  $n = 4$ , mean  $\pm$  standard deviation).

K<sup>+</sup> permeability in these HKT1 proteins is attributed to a critical glycine residue which resides in the K<sup>+</sup> channel selectivity filter GYG motif (Mäser et al. 2002b). Nevertheless, genetic evidence has been shown that HKT1 negatively regulates K<sup>+</sup> nutrition in *Arabidopsis* (Rus et al. 2004), despite the fact that *Arabidopsis* HKT1 selectively transports Na<sup>+</sup> but not K<sup>+</sup> (Uozumi et al. 2000). Our results support the negative role of HKT1 in K<sup>+</sup> nutrition (Fig. 5C and D). With GB03 exposure, plants accumulate more K<sup>+</sup> in roots where *HKT1* expression is downregulated. On the other hand, GB03-exposed plants accumulate more *HKT1* transcripts as well as less K<sup>+</sup> in shoots. Therefore, regulation of *HKT1* expression by GB03 modulates not only Na<sup>+</sup> but also K<sup>+</sup> homeostasis tissue specifically. Compared with the control plants, GB03-exposed plants show a higher K<sup>+</sup>/Na<sup>+</sup> ratio in roots whereas no difference is observed in shoot K<sup>+</sup>/Na<sup>+</sup> ratios. As a result, GB03-exposed plants have greater capacity for K<sup>+</sup>/Na<sup>+</sup>-selective accumulation from a whole-plant perspective, in medium supplemented with 100 mM NaCl.

The plant symbiont GB03 displays an as yet unidentified volatile signal or signals to achieve tissue-specific HKT1 regulation. In addition, GB03-generated volatile signals that induce salt tolerance are produced whether the bacteria are grown under low or elevated NaCl (0, 100, and 150 mM) in the media (data not shown). HKT-type transporters have been found in several plant species, including rice and wheat, yet regulatory mechanisms of gene expression remains unknown (Fairbairn et al. 2000; Garciasdeblas et al. 2003; Horie et al. 2001; Ren et al. 2005; Rubio et al. 1995). Future analyses of volatile chemicals from GB03 that trigger HKT1 regulation and regulatory elements of *HKT1* expression in planta are likely to reveal molecular mechanisms for the tissue-specific regulation of *HKT1*.

## MATERIALS AND METHODS

### Plant materials and treatments.

*Arabidopsis thaliana* seed were surface sterilized and then planted on one side of specialized plastic petri dishes (100 by

15 mm) that contained a center partition (I plates; Fisher Scientific, Pittsburgh, U.S.A.); both sides contained half-strength Murashige and Skoog (MS) solid media with 0.8% (wt/vol) agar, 1.5% (wt/vol) sucrose, and NaCl. Half-strength MS media was prepared according to Murashige and Skoog (1962). Before being placed in the growth room, seed were vernalized for 2 days at 4°C with an absence of light. The growth room was set to a cycle of 16 h of light and 8 h of darkness under metal halide and high-pressure sodium lamps with a total light intensity of 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , a temperature of  $21 \pm 4^\circ\text{C}$ , and a relative humidity of  $40 \pm 10\%$ .

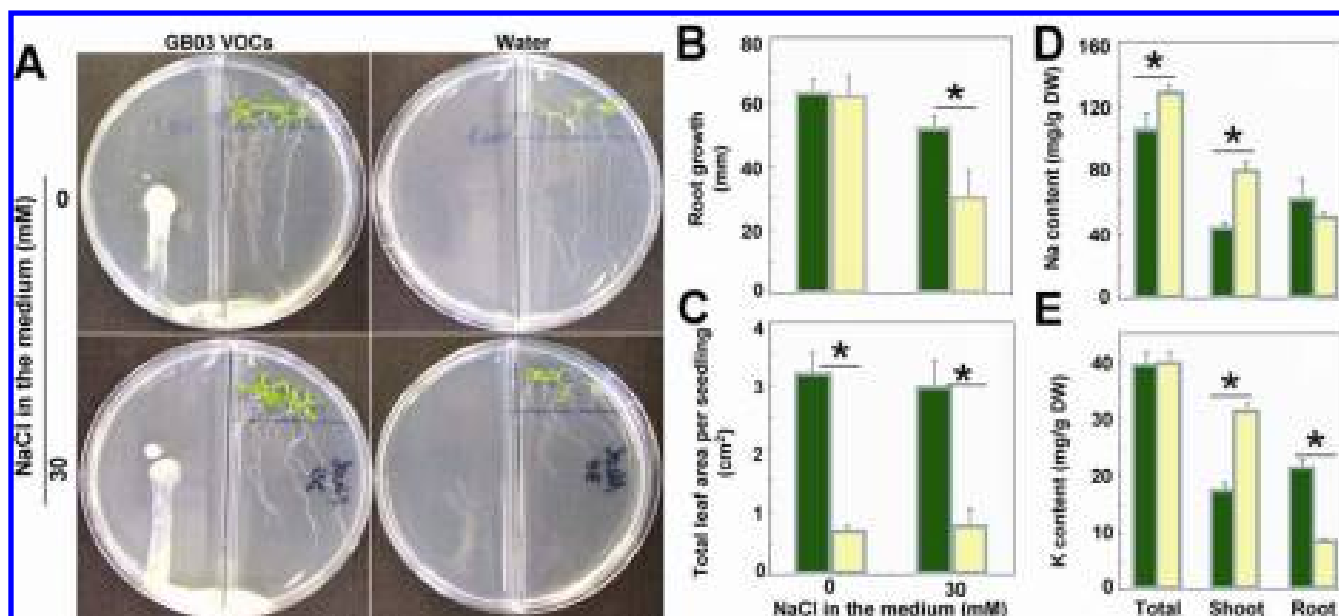
One day before plant experiments, bacterial strains were streaked onto tryptic soy agar (TSA) plates and incubated at 28°C in the absence of light for 24 h. PGPR cells were harvested from TSA plates in double-distilled water (DDW) to yield  $10^9$  CFU  $\text{ml}^{-1}$ , as determined by optical density and serial dilutions with plate counts. Two-day-old *Arabidopsis* seedlings then were inoculated with 20  $\mu\text{l}$  of bacterial suspension culture or DDW applied drop wise to the nonplant side of the petri dish.

### Quantification of leaf surface and root growth.

For leaf area quantification, seedlings were photographed 14 days after the treatments using an Olympus C-4000 camera (Olympus American Inc., Melville, NY, U.S.A.). Images were imported into Adobe Photoshop 5.5 and leaf surface area measurements were determined by using an available histogram function; pixel areas were calibrated based the total petri dish area. For root growth measurement, *sos3* seed were germinated and grown in half-strength MS media without supplement of NaCl. Four-day-old seedlings were transferred to vertical I-plates containing 0 or 30 mM NaCl in the media for plant growth. Root growth measurement was performed 5 days after transfer.

### Na<sup>+</sup> and K<sup>+</sup> content measurement.

Plants with 14 days of GB03 or water exposure were harvested, rinsed with deionized water, and dried at 65°C for 2 days. Dried tissue was extracted with 100% HNO<sub>3</sub> overnight,



**Fig. 6.** GB03 suppresses sodium hypersensitivity for *sos3*. GB03 attenuates root growth inhibition with elevated NaCl by **A**, visual inspection and **B**, root growth quantification ( $n = 30$ ). **C**, GB03-exposed *sos3* plants also show improved shoot growth with 30 mM NaCl ( $n = 36$ ). Seed of *sos3* were germinated and grown in half-strength Murashige and Skoog media without NaCl for 4 days and compared 5 days after transfer to specified salt media. **D**, GB03 reduces Na<sup>+</sup> accumulation in *sos3* plants and, specifically, in shoots. **E**, K<sup>+</sup> levels in shoots and roots of *sos3* plants were decreased and increased by GB03, respectively. Measurements for Na<sup>+</sup>, K<sup>+</sup>, and total leaf area were performed 14 days post-treatment. Green and yellow bars represent GB03 and water control treatments, respectively; an asterisk (\*) indicates  $P \leq 0.05$  for treated versus controls ( $t$  test; mean  $\pm$  standard deviation).

followed by incubation at 90 to 100°C for 1 h. Then, 2.5% HNO<sub>3</sub> was added to the digested samples. Aqueous Na<sup>+</sup> was determined by atomic absorption spectrophotometry (Model 6300; Shimadzu Scientific Instruments, Columbia, MD, U.S.A.).

### RNA extraction and reverse-transcriptase polymerase chain reaction.

RNA was extracted following Qiagen RNeasy protocol (Qiagen, Valencia, CA, U.S.A.).

**Reverse transcriptase polymerase chain reaction.** Whole-plant RNA was extracted 4 days after GB03 or H<sub>2</sub>O exposure. First-strand cDNA was synthesized from 5 µg of total RNA using MuMLV-RT (Fisher Scientific, Houston). The following primers were used (5' to 3'): *UBQ10*, CGATTACTCTTGAGG TGGAG and AGACCAAGTGAAGTGTGGAC; and *HKT1*, ATCGCTGTGACGTTGAGACT and ATCAGAAACCCTAGC ACCGT. Agarose gel electrophoresis images were taken by Kodak Gel Logic 100 Imaging System (Fisher Scientific, Houston) and quantified by using Image J 1.33u.

**Quantitative real-time polymerase chain reaction.** RNA from shoots and roots were extracted separately at 1, 2, 3, and 4 days postexposure. Reverse transcription of all the RNA samples was carried out using random hexamers. ABI TaqMan reverse transcription reagent kit (Applied Biosystems, Foster City, CA, U.S.A.) was used for first-strand synthesis in 50-µl reactions (containing 1 µg of total RNA) at 37°C for 60 min. The expression levels of the *HKT1* gene in different *Arabidopsis* tissues were detected by the ABI PRISM 7000 sequence detection system. The primers were designed by using the PrimerQuest program (Integrated DNA Technology, Coralville, IA, U.S.A.). 18S rRNA primers were used for RNA normalization. SYBR Green PCR master mix (Applied Biosystems) was used for 50-µl polymerase chain reaction (PCR) reactions as follow: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 60 s. Each sample was assayed three times. The relative expression levels of all the samples were calculated and analyzed (User Bulletin no. 2, ABI PRISM 7700 sequence detection system). The threshold cycle (Ct value) of the target genes and 18S RNA in different samples were obtained after quantitative real-time PCR reaction. In brief, the normalizer 18S RNA Ct value was subtracted from the gene of interest Ct (target gene) to produce the dCt value of the sample. The dCt value of the calibrator (the sample with the highest dCt value) was subtracted from every other sample to produce the ddCt value. Two to the -ddCt power (2<sup>-ddCt</sup>) was taken for every sample as the relative expression levels.

### ACKNOWLEDGMENTS

Financial support was provided by the Welch (Grant D-1478) and ACS-Frasch Foundations for Chemical Research.

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